Two distinct arginine methyltransferases are required for biogenesis of Sm-class ribonucleoproteins

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Small nuclear ribonucleoproteins (snRNPs) are core components of the spliceosome. The U1, U2, U4, and U5 snRNPs each contain a common set of seven Sm proteins. Three of these Sm proteins are posttranslationally modified to contain symmetric dimethylarginine (sDMA) residues within their C-terminal tails. However, the precise function of this modification in the snRNP biogenesis pathway is unclear. Several lines of evidence suggest that the methyltransferase protein arginine methyltransferase 5 (PRMT5) is responsible for sDMA modification of Sm proteins. We found that in human cells, PRMT5 and a newly discovered type II methyltransferase, PRMT7, are each required for Sm protein sDMA modification. Furthermore, we show that the two enzymes function nonredundantly in Sm protein methylation. Lastly, we provide in vivo evidence demonstrating that Sm protein sDMA modification is required for snRNP biogenesis in human cells.

Introduction

Small nuclear RNPs (snRNPs) are core components of spliceosomes and are required for the catalytic steps of splicing. Most spliceosomal snRNPs, with the exception of U6, contain a common set of Sm proteins that associate with the Sm site of the small nuclear RNA (snRNA; Matera et al., 2007). The biogenesis of Sm-class snRNPs is a highly orchestrated process that takes place in multiple cellular compartments (Matera and Shpargel, 2006). Subsequent to transcription and nuclear export of the snRNA, the survival of motor neurons (SMN) complex mediates the assembly of snRNPs by loading Sm proteins onto the snRNA (Meister et al., 2002; Paushkin et al., 2002). The core factor within this large oligomeric complex is the SMN protein (Meister et al., 2002; Paushkin et al., 2002). Importantly, mutations that reduce the level of SMN protein result in the inherited human neuromuscular disorder spinal muscular atrophy (Lefebvre et al., 1995). The molecular etiology of spinal muscular atrophy is currently unknown. Recent work suggests that the perturbation of snRNP biogenesis may be a contributing factor (Winkler et al., 2005). However, these findings do not rule out tissue-specific functions of SMN as factors contributing to the

disease pathology (Briese et al., 2005; Eggert et al., 2006; Rajendra et al., 2007).

Three of the seven Sm proteins, B/B′, D1, and D3, contain symmetric dimethylarginine (sDMA) modifications within their C-terminal tails (Brahms et al., 2000, 2001). This posttranslational modification is catalyzed by type II protein arginine methyltransferases (PRMTs; for review see Bedford and Richard, 2005). Type I enzymes catalyze the more common asymmetric dimethylarginine (aDMA) modification. PRMT5 and PRMT7 have each been shown to possess type II methyltransferase activity and to symmetrically dimethylate Sm proteins in vitro (Branscombe et al., 2001; Rho et al., 2001; Lee et al., 2005). Reduction of PRMT5 levels using RNAi correlates with a decrease in the level of Sm protein sDMA modification (Boisvert et al., 2002). Furthermore, in cytoplasmic lysates, PRMT5 is found in a complex with MEP50/WD45, iCln, and Sm proteins (Friesen et al., 2001b, 2002; Meister et al., 2001). PRMT7 was identified more recently as a type II methyltransferase (Lee et al., 2005). Consequently, very little is known about this enzyme.

The precise role of Sm protein sDMA modification in snRNP biogenesis remains unclear. Recruitment of Sm proteins to the SMN complex is thought to be facilitated by sDMA modification. Consistent with this notion, SMN binds with a much higher affinity to sDMA-modified Sm proteins (Brahms et al., 2001; Friesen et al., 2001a). However, a loss of function mutation in *dart5*, the fly orthologue of PRMT5, is homozygous viable (Gonsalvez et al., 2006). The mutants displayed no overt

Supplemental Material can be found at:
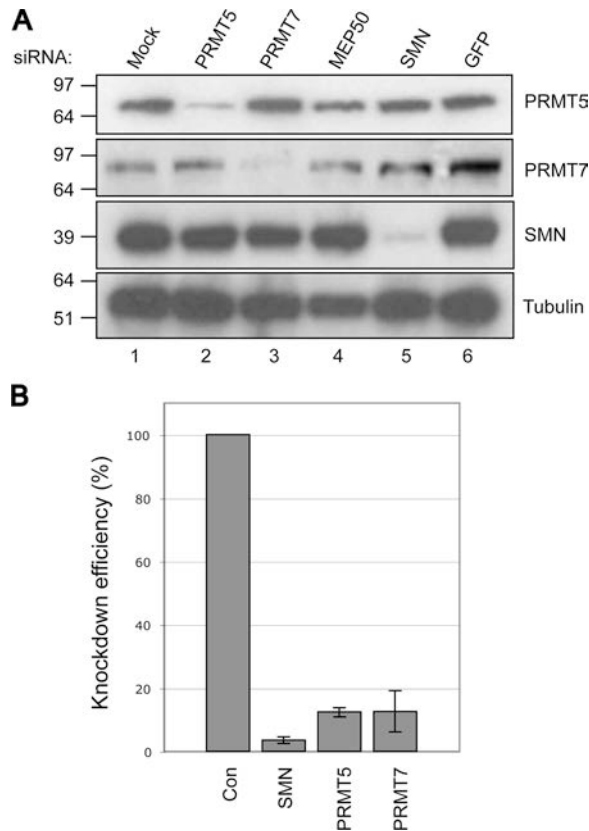http://jcb.rupress.org/content/suppl/2007/08/20/jcb.200702147.DC1.html

Figure 1.   **siRNA treatment of PRMT5, PRMT7, MEP50, and SMN.** (A) HeLa cells were transfected with siRNAs targeting PRMT5 (lane 2), PRMT7 (lane 3), MEP50 (lane 4), and SMN (lane 5). As a control, cells were untransfected (mock; lane 1) or transfected with siRNAs against GFP (lane 6). 72 h after transfection, lysates were prepared and probed with the indicated antibodies. (B) The protein levels from three separate experiments were quantified. The protein levels were normalized to tubulin and graphed as a fraction of the mock transfection. Error bars represent SD.

defect in snRNP levels despite expressing Sm proteins that were not recognized by two sDMA-specific antibodies, SYM10 and Y12 (Gonsalvez et al., 2006). To gain a better understanding of the role of Sm protein sDMA modification in snRNP biogenesis in humans, we depleted HeLa cells of PRMT5 or PRMT7 using RNAi. Surprisingly, we found that both PRMT5 and PRMT7 were required for efficient Sm protein sDMA modification. Both enzymes independently associated with Sm proteins but not with each other. In addition, we demonstrate that PRMT5 and PRMT7 do not function in an additive or redundant manner, thus suggesting a unique requirement for each methyltransferase in the Sm protein methylation pathway. Finally, we show that the symmetric dimethylation of Sm proteins is required for cytoplasmic snRNP assembly in human cells.

Results and discussion

PRMT5 and PRMT7 are required for Sm protein symmetric dimethylation

To understand the specific requirement for Sm protein sDMA modification in mammals, we examined the in vivo functions of PRMT5, MEP50, and PRMT7. HeLa cells were depleted of these proteins using RNAi (Fig. 1, A and B). siRNAs targeting SMN

or GFP were used as controls (Fig. 1, A and B). The siRNAs directed against PRMT5, PRMT7, and SMN were able to deplete >80% of their respective target proteins (Fig. 1 B). Treatment of cells with siRNAs targeting MEP50, a PRMT5 complex member, caused a slight codepletion of PRMT5 (Fig. 1 A). This finding is consistent with our previous results in *Drosophila melanogaster*: the mutation of *valois* resulted in a loss of Dart5 expression (Gonsalvez et al., 2006). In contrast, only specific siRNA treatments reduced the level of PRMT7 (Fig. 1 A).

We next analyzed the methylation status of Sm proteins in the depleted lysates using the sDMA-specific antibodies SYM10, SYM11, and Y12 (Fig. 2 A). Unmodified and asymmetrically dimethylated Sm proteins are not recognized by these antibodies (Brahms et al., 2000; Boisvert et al., 2002, 2003). Consistent with previous findings (Boisvert et al., 2002), the knockdown of PRMT5 resulted in a reduction in Sm protein sDMA modification (Fig. 2 A, lane 2). A similar effect was also observed when cells were treated with siRNAs targeting MEP50 (Fig. 2 A, lane 4). However, because MEP50 RNAi treatment codepletes PRMT5, we cannot conclude whether this defect in methylation is direct. Curiously, we found that PRMT7 knockdown also caused a reduction in Sm protein sDMA modification (Fig. 2 A, lane 3). Because the depletion of PRMT7 does not codeplete PRMT5 (Fig. 1 A), this effect is likely to be direct.

In addition to Sm proteins, SYM10 and SYM11 also recognize several uncharacterized sDMA-modified proteins (Boisvert et al., 2002, 2003). The same SYM10- and SYM11-reactive proteins were hypomethylated in the PRMT5 and MEP50 siRNA–treated lysates (Fig. 2 A). This finding was expected because PRMT5 and MEP50 are part of the same complex and also because the depletion of MEP50 codepletes PRMT5. In contrast, PRMT7 siRNA treatment resulted in the hypomethylation of only a subset of these proteins (Fig. 2 A). Thus, PRMT5 and PRMT7 do not always act on the same substrates in vivo. Consistently, we observed that the protein–protein interaction profile of PRMT5 and PRMT7 was largely nonoverlapping (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200702147/DC1).

We next determined whether Sm proteins were sDMA modified in an additive manner by depleting cells of both PRMT5 and PRMT7 (Fig. 2 B). Interestingly, double depletion did not disrupt Sm protein sDMA modification to a greater extent than either single depletion alone. Thus, the two enzymes do not function additively to produce the full complement of methylated Sm proteins. We also attempted to determine whether PRMT5 and PRMT7 could functionally compensate for each other with respect to Sm protein sDMA modification (Fig. 2 C). We found that the overexpression of PRMT7 was not able to restore sDMA modification of the Sm protein in cells that were depleted of PRMT5. Thus, PRMT7 cannot functionally substitute for PRMT5. At this time, however, we cannot conclude whether the converse is also true. Although cells depleted of PRMT7 alone show relatively little cytotoxicity, they do not survive the subsequent DNA transfection procedure. Transfection of PRMT7 siRNA–treated cells with empty vector or a PRMT5-expressing plasmid resulted in rapid and pronounced cell death.
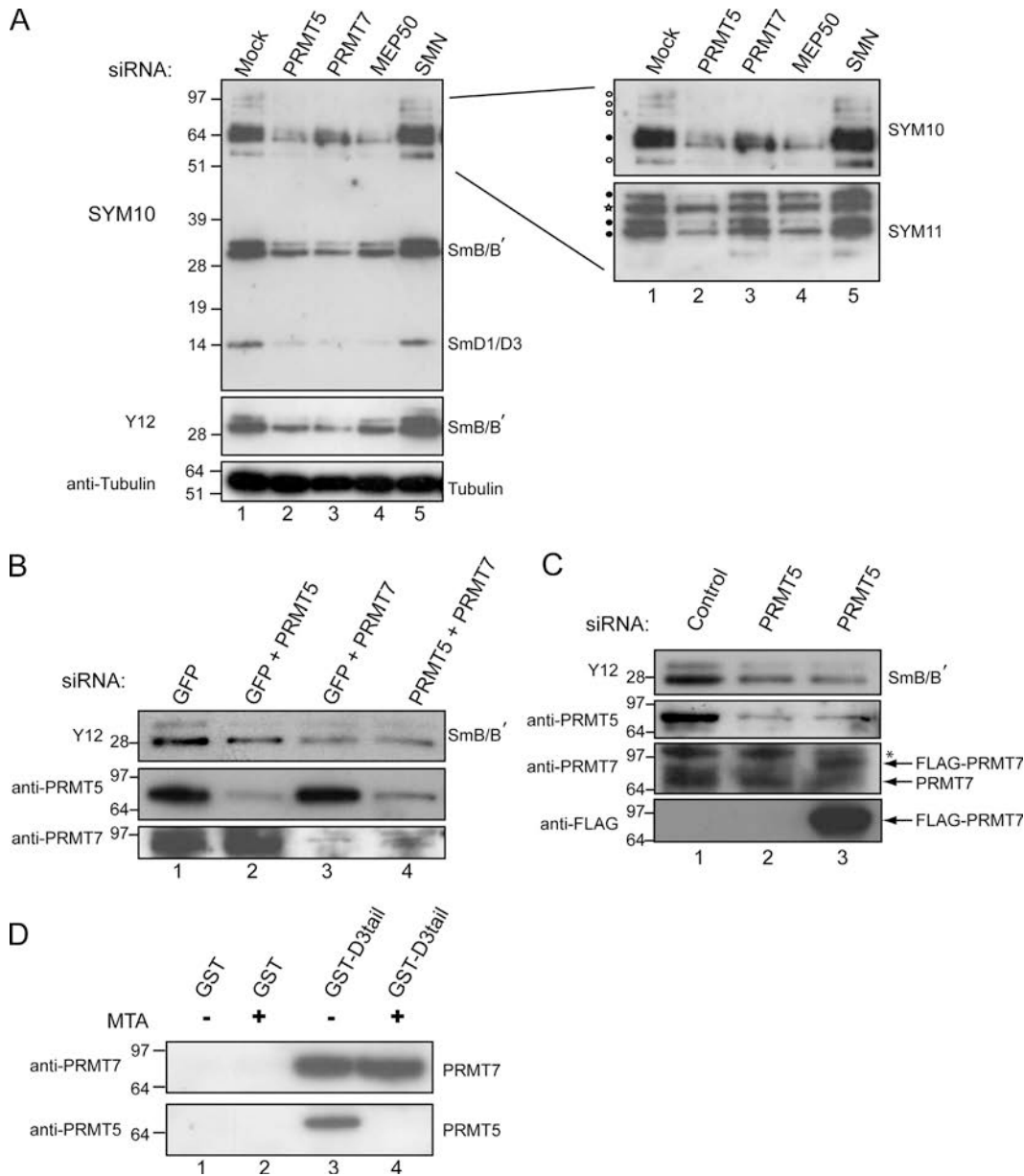
Figure 2.   **PRMT5 and PRMT7 are required for sDMA modification of Sm proteins.** (A) Lysates from mock-treated (lane 1) and PRMT5 (lane 2), PRMT7 (lane 3), MEP50 (lane 4), and SMN (lane 5) siRNA–treated cells were probed with anti-sDMA antibodies SYM10 (top), Y12 (middle), and SYM11 (bottom right). The lysates were probed with tubulin to verify load (bottom left). The open circles represent proteins that require PRMT5, MEP50, and PRMT7 for their methylation. The closed circles represent proteins that require PRMT5 and MEP50 but not PRMT7 for their methylation. The star represents a protein whose methylation status appears unaffected upon the depletion of PRMT5, MEP50, or PRMT7. (B) HeLa cells were treated with siRNAs against PRMT5 (lane 2), PRMT7 (lane 3), or both (lane 4). GFP siRNAs were used as a control (lanes 1–3). Lysates were prepared and analyzed by blotting with the indicated antibodies. (C) HeLa cells were depleted of PRMT5 (lanes 2 and 3). GFP siRNAs were used as a control (lane 1). A FLAG-PRMT7 overexpression construct was transfected into PRMT5-depleted cells (lane 3). Empty vector was transfected as a control (lanes 1 and 2). Lysates were prepared and analyzed by blotting with the indicated antibodies. The asterisk represents a protein that cross reacts with the PRMT7 antibody. (D) 1 μg GST (lanes 1 and 2) or GST-SmD3 C-terminal tail (GST-D3tail; lanes 3 and 4) immobilized on glutathione–Sepharose beads was incubated for 1 h with total HeLa lysates from untreated cells (lanes 1 and 3) or cells treated with MTA (750 μM for 24 h; lanes 2 and 4). The bound proteins were eluted by boiling in SDS buffer and analyzed by blotting using the indicated antibodies.

PRMT5 has been shown to associate with the C-terminal arginine-glycine (RG)–rich tail of SmD3 (Friesen et al., 2001b; Meister et al., 2001; Meister and Fischer, 2002). Therefore, we tested for a similar association between SmD3 and PRMT7. We found that the GST-tagged C terminus of SmD3 (GST-D3tail) was able to specifically purify both PRMT5 and PRMT7 from cell lysates (Fig. 2 D). Interestingly, the association between

PRMT7 and GST-D3tail was unaffected by prior treatment of the cells with the methyltransferase inhibitor 5′-deoxy-5′-(methylthio)adenosine (MTA; Fig. 2 D). In contrast, a similar treatment disrupted the association between PRMT5 and GST-D3tail (Fig. 2 D). In addition to GST-D3tail, PRMT7 is able to bind to full-length GST-SmD3 and -SmB (unpublished data). Because PRMT7 is able to associate with Sm proteins but not

Figure 3. **sDMA modification of Sm proteins is required for efficient snRNP biogenesis.** (A) HeLa cells stably expressing CFP-SmB were depleted of PRMT5 (lane 3) or PRMT7 (lane 4) using RNAi. Mock-treated cells were used as a control (lanes 1 and 2). 72 h after transfection, the cells were harvested, cytoplasmic fractions were prepared, and CFP-SmB was immunoprecipitated using polyclonal GFP antibodies (lanes 2–4). To verify binding specificity, a control myc antibody was used (lane 1). The level of coprecipitating SMN was examined (top). The immunoprecipitation efficiency was verified using a monoclonal GFP antibody (bottom). (B) Cells treated for 24 h with either 750 µM MTA (lane 3) or DMSO (lanes 1 and 2) were pulse labeled with [^{35}S]methionine and [^{35}S]cysteine for 1 h. Subsequently, complete media was added to the cells for a 30-min chase. The cells were harvested, and snRNPs were immunoprecipitated using anti-TMG antibody–coated beads (lanes 2 and 3). Protein A beads were used to demonstrate binding specificity (lane 1). Representative samples were also probed with the tubulin antibody (bottom) to serve as a loading control. The abundant copurifying U1 snRNP proteins are indicated. (C) Cells depleted of SMN (lane 3), PRMT5 (lane 4), or PRMT7 (lane 5) were used in the pulse-chase assay described in A using similar controls. (D) The precipitated levels of the abundant snRNP proteins from three separate RNAi/snRNP assembly experiments were quantified and graphed as a fraction of the mock transfection. Error bars represent SD. (E) HeLa cells stably expressing CFP-SmB were depleted of SMN (lane 3), PRMT5 (lane 4), or PRMT7 (lane 5). Mock-treated cells were used as a control (lanes 1 and 2). The cells were pulsed with [^{32}P]orthophosphate for 2 h. The cells were harvested, total lysates were prepared, and CFP-SmB was immunoprecipitated. Protein A beads were used to demonstrate binding specificity (lane 1). The associated snRNAs were extracted and examined by autoradiography.
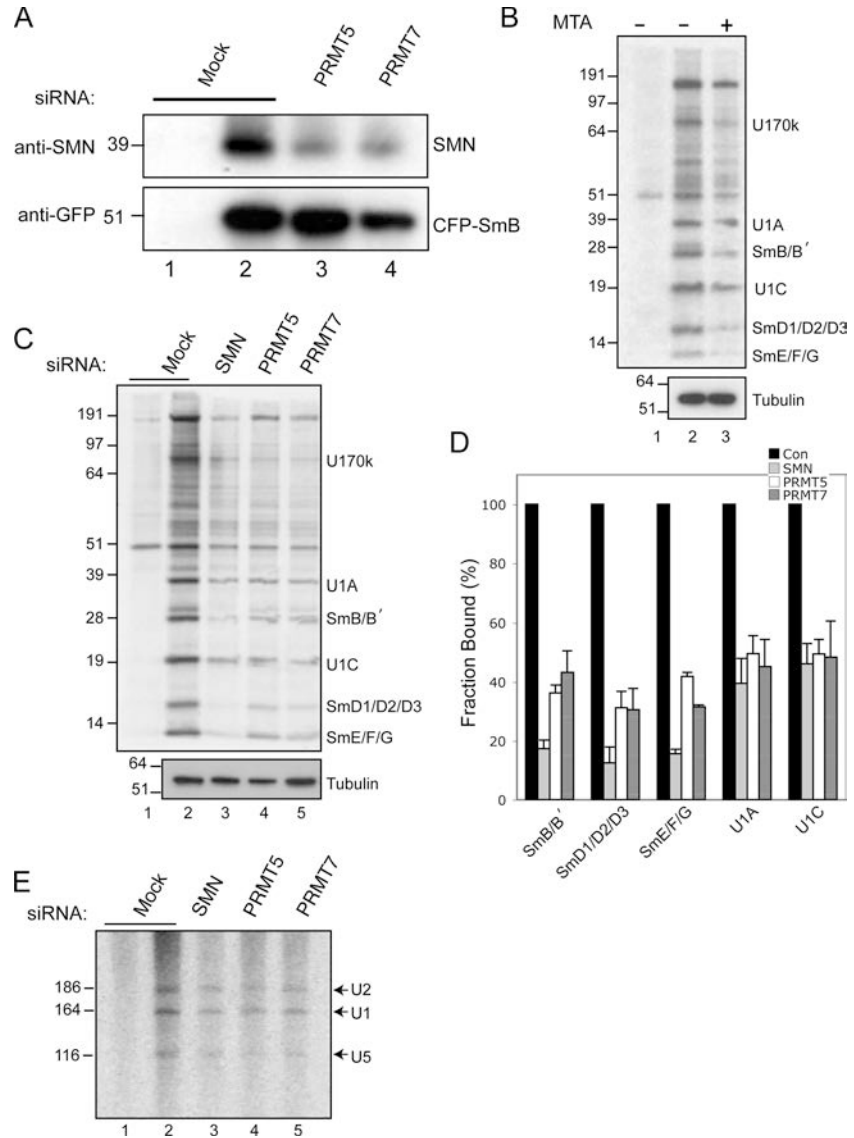
with PRMT5 (Lee et al., 2005), these observations suggest a direct role for PRMT7 in Sm protein methylation.

Cytoplasmic snRNP assembly requires the activities of both PRMT5 and PRMT7

During the cytoplasmic phase of snRNP biogenesis, the SMN complex loads Sm proteins onto the Sm sites of snRNAs (Meister et al., 2002; Paushkin et al., 2002). SMN has a much higher affinity for sDMA-modified Sm proteins in comparison with unmodified or aDMA-modified Sm proteins (Brahms et al., 2001; Friesen et al., 2001a). Consistent with the finding that both enzymes are required for the sDMA modification of Sm proteins, both siRNA treatments interfered with the SMN–Sm interaction (Fig. 3 A). Previous studies demonstrated that the SMN–Sm interaction was disrupted by treatment with general methyltransferase inhibitors (Brahms et al., 2001; Friesen et al., 2001a). Our current results extend these findings and demonstrate that the specific depletion of either PRMT5 or PRMT7 is able to mimic the drug treatment.

To determine whether the methylation of Sm proteins is a prerequisite for efficient snRNP assembly, we treated HeLa cells with MTA. Subsequently, a pulse-chase experiment using a mixture of [^{35}S]methionine and [^{35}S]cysteine was used to examine the in vivo kinetics of snRNP assembly. The newly assembled snRNPs were immunoprecipitated using anti-trimethylguanosine (TMG)–coated beads. Anti-TMG antibodies recognize the cap structure present on mature Sm-class snRNPs. More precisely, this assay monitors the step in snRNP biogenesis that is immediately downstream of Sm core assembly (Matera et al., 2007). Interestingly, we found that MTA treatment substantially disrupted snRNP assembly (Fig. 3 B), suggesting that methylation is required for efficient snRNP biogenesis. Because MTA treatment reduced but did not completely abolish Sm protein sDMA modification (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200702147/DC1), we conclude that the residual snRNPs assembled under these conditions contain methylated Sm proteins. To specifically narrow down the snRNP assembly defect to sDMA modification of Sm proteins, HeLa cells depleted of
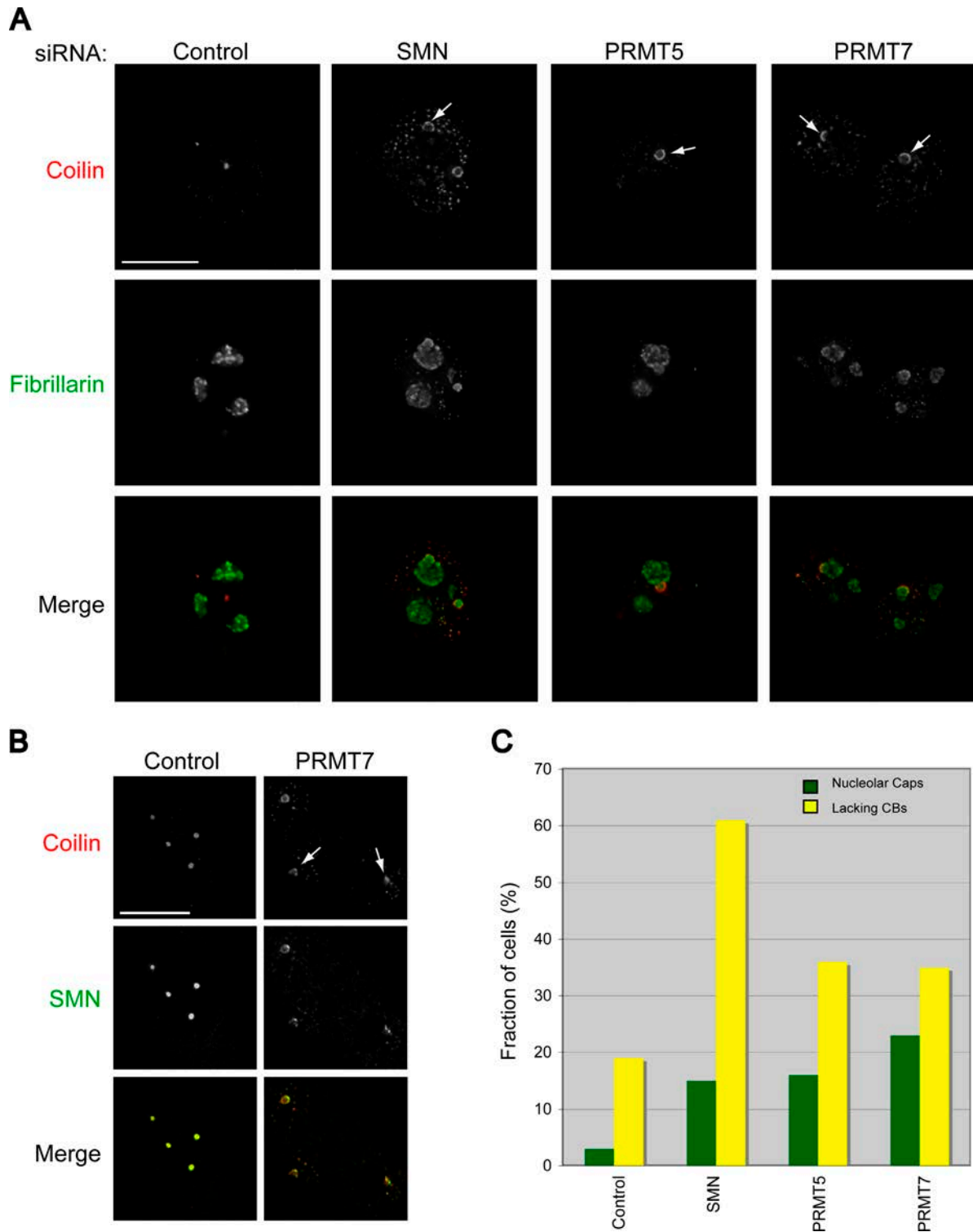
Figure 4. **Coilin is found in nucleolar caps in cells depleted of SMN, PRMT5, or PRMT7.** (A) Control cells as well as cells depleted of SMN, PRMT5, or PRMT7 were processed for immunofluoresence using antibodies against coilin (red) and fibrillarin (green). The arrows indicate the nucleolar capping phenotype. The images in A and B represent the maximum projection of a z stack that has been deconvolved. (B) Cells depleted of PRMT5 and PRMT7 were processed for immunofluoresence using antibodies against coilin (red) and SMN (green). PRMT7-depleted cells are shown. Note that the coilin nucleolar caps (arrows) also contain SMN. (C) Cells depleted of SMN, PRMT5, or PRMT7 were scored for the presence of CBs and also for the coilin nucleolar capping phenotype. The results were graphed as a percentage of the mock-treated cells. A total of 200 cells were scored for each treatment condition. Bars, 10 μM.

PRMT5 or PRMT7 were used in the pulse-chase assay. HeLa cells treated with siRNAs targeting SMN served as a control. Consistent with previous results (Shpargel and Matera, 2005; Wan et al., 2005; Winkler et al., 2005), the depletion of SMN severely disrupted snRNP assembly (Fig. 3, C and D; and Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200702147/DC1).

Figure 5. **PRMT5, PRMT7, and SMN depletion affects cytoplasmic snRNP assembly.** (A) Cells treated for 24 h with either 750 μM MTA (lanes 2 and 4) or DMSO (lanes 1 and 3) were pulse labeled with ^{35}S as described in Fig. 3 A. The cells were harvested, and nuclear and cytoplasmic fractions were prepared. The snRNPs from each fraction were immunoprecipitated using anti-TMG antibody–coated beads. (B) Cells depleted of SMN (lanes 2 and 6), PRMT5 (lanes 3 and 7), or PRMT7 (lanes 4 and 8) were used in the pulse-chase assay. Mock-treated cells were used as a control (lanes 1 and 5). Binding conditions are the same as in A.
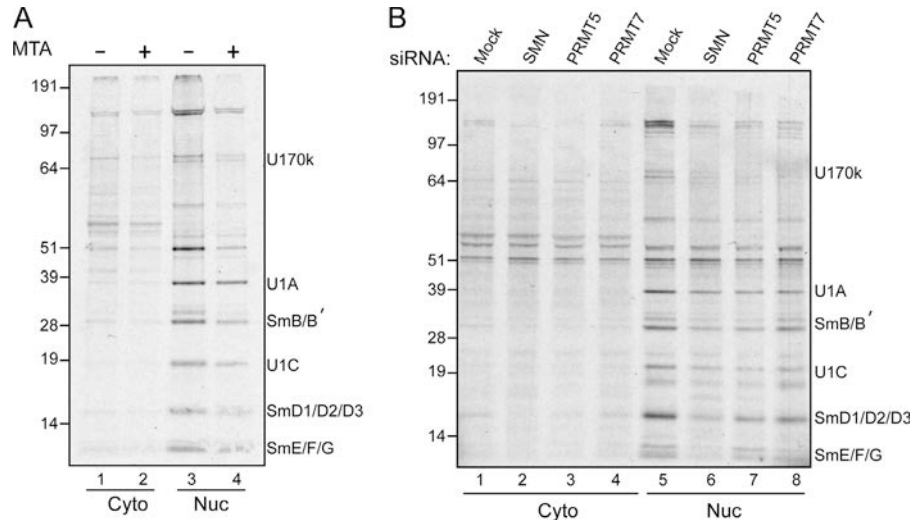
A similar disruption of snRNP assembly was also observed in cells depleted of either PRMT5 or PRMT7 (Figs. 3, C and D; and S3). It is worth noting that snRNP assembly was disrupted to a somewhat lesser extent upon the depletion of either PRMT5 or PRMT7 as compared with SMN (Fig. 3, C and D). These findings are consistent with the observation that PRMT5 and PRMT7 depletion reduces but does not eliminate the SMN–Sm interaction (Fig. 3 A).

The most likely function for PRMT5 and PRMT7 in the snRNP biogenesis pathway is to enable the efficient association of SMN with Sm proteins. Therefore, we directly examined the assembly of Sm cores in control or depleted lysates. For this experiment, a HeLa strain stably expressing CFP-SmB (Sleeman et al., 2001) was used. The cells were pulsed with [^{32}P]orthophosphate. Subsequently, tagged SmB was immunoprecipitated from the labeled lysates. The associated RNAs were examined by autoradiography. As expected, the depletion of SMN considerably inhibited Sm core assembly (Fig. 3 E). Likewise, the depletion of either PRMT5 or PRMT7 also resulted in a similar Sm core assembly defect (Fig. 3 E).

Sm protein sDMA modification is primarily required for cytoplasmic snRNP assembly

The depletion of core snRNP assembly factors results in the breakdown of Cajal bodies (CBs) and the redistribution of coilin to the nucleolus (Shpargel and Matera, 2005; Girard et al., 2006; Lemm et al., 2006). Therefore, we examined whether the depletion of PRMT5 or PRMT7 also leads to CB breakdown (Fig. 4). Consistent with these earlier studies, CBs were undetected in ~60% of SMN-depleted cells (Fig. 4 C). In contrast, roughly 30% of cells depleted of PRMT5 or PRMT7 lacked visible CBs (Fig. 4 C). Also consistent with these earlier studies, coilin localized within the nucleolus in a subset of SMN-depleted cells (unpublished data). This phenotype was not observed in cells depleted of PRMT5 or PRMT7. However, in a subset of cells depleted for PRMT5, PRMT7, or SMN, coilin localized at the nucleolar periphery (Fig. 4, A and C). Interestingly, in cells depleted of PRMT5 or PRMT7, we found that these nucleolar caps also contained SMN (Fig. 4 B and not depicted). Thus, in contrast to SMN depletion, PRMT5 or PRMT7 depletion produced

a milder CB phenotype. One explanation for this finding is that SMN depletion results in a more severe snRNP defect than either methyltransferase deletion (Fig. 3 D). Alternatively, in contrast to PRMT5 and PRMT7, SMN may play additional roles in targeting imported snRNPs to CBs (Ospina et al. 2005).

Finally, we tested whether Sm protein methylation was required for snRNP import using the ^{35}S pulse-chase assay. Labeled cells were harvested, and nuclear and cytoplasmic fractions were prepared. Each fraction was then subjected to immunoprecipitation using anti-TMG antibody–coated beads. TMG-positive RNPs produced during the 1.5-h pulse-chase period were nearly all located in the nuclear fraction (Fig. 5 A), suggesting that in HeLa cells, snRNP biogenesis and nuclear import occur relatively rapidly. As observed previously (Fig. 3 A), MTA treatment disrupted snRNP assembly. However, the residual snRNPs that were assembled were present almost exclusively in the nuclear fraction (Fig. 5 A). Similar to MTA treatment, RNAi-mediated depletion of SMN, PRMT5, or PRMT7 resulted in snRNP assembly defects (Fig. 5 B). As with MTA treatment, the residual snRNPs that were assembled were also imported into the nucleus (Fig. 5 B). Thus, sDMA modification of Sm proteins does not play a major role in the nuclear import of snRNPs.

During the preparation of this manuscript, PRMT9 was shown to possess type II methyltransferase activity and to methylate a variety of targets in vitro, including SmB (Cook et al., 2006). Therefore, it will be interesting to determine whether PRMT9 plays a role in Sm protein methylation and snRNP biogenesis in vivo. In addition, CARM1/PRMT4, a type I methyltransferase, was recently shown to asymmetrically dimethylate SmB in vivo (Cheng et al., 2007). Because aDMA residues are found exclusively on nuclear Sm proteins (Miranda et al., 2004), this modification is not likely to be required for the cytoplasmic phase of snRNP assembly. The aDMA modification of Sm proteins may be important for subnuclear targeting of snRNPs or for the regulation of pre-mRNA splicing (Cheng et al., 2007).

In conclusion, we have shown that two distinct methyltransferases, PRMT5 and PRMT7, are required for Sm protein sDMA modification and snRNP assembly. We envision that both enzymes function in the snRNP pathway by sDMA modification

of Sm proteins, thus increasing their affinity for the SMN complex. A previous study showed that the activity of the SMN complex in Sm core assembly is stimulated by phosphorylation (Grimmler et al., 2005). Here, we demonstrate that sDMA modification of Sm proteins also serves an important regulatory function. Thus, mammalian snRNP biogenesis is controlled by multiple posttranslational events. *Drosophila* may differ from mammals in this regard. Whereas PRMT5 is required for snRNP biogenesis in human cells, the loss of Dart5 does not result in decreased snRNP levels in *Drosophila*. However, flies also express an orthologue of PRMT7 called Dart7. Therefore, it will be interesting to test whether snRNP assembly in *Drosophila* is independent of Sm protein sDMA modification.

Materials and methods

DNA/siRNA constructs
GST-tagged SmD3 C-terminal tail (GST-D3tail) was a gift from G. Dreyfuss (University of Pennsylvania, Philadelphia, PA). The FLAG-PRMT7 overexpression plasmid was constructed by cloning PRMT7 cDNA into the p3X-FLAG-myc-CMV-23 expression vector (Sigma-Aldrich). Because the PRMT7 construct contains its endogenous stop codon, the C-terminal myc tag is not translated. The siRNAs used in these studies were obtained from Ambion. The PRMT5 siRNA sequence is GGCCAUCUAUAAAUGUCUG, and the PRMT7 siRNA sequence is GCUAUUUCCCAUCCACGUG.

Cell culture and transfections
HeLa cells were cultured in DME supplemented with 10% FBS. For each RNAi transfection, 275 pmol siRNAs were transfected into a 20% confluent T25 flask using the DharmaFECT1 reagent (Dharmacon). For those experiments in which the steady-state methylation status of the Sm proteins was analyzed (Fig. 2, A–C), the cells were treated with siRNA twice—once on day 1 and once on day 3. The cells were harvested and analyzed on day 5. This was done to accommodate the long half-life of Sm proteins. For the rest of the RNAi experiments, the cells were treated with siRNA just once. The cells were harvested and analyzed on day 3. DNA was transfected using Effectene (QIAGEN).

Lysate preparation
HeLa lysates were prepared by resuspending the cells in radioimmunoprecipitation assay buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1% NP-40, and 1 mM EDTA) containing protease inhibitors (Halt protease inhibitor cocktail kit; Pierce Chemical Co.) and passing several times through a 25-gauge needle. The lysate was cleared by centrifugation at 10,000 g for 5 min at 4°C. Nuclear and cytoplasmic HeLa fractions were prepared using the N-PER fractionation kit (Pierce Chemical Co.) as directed. Bacterial lysates were prepared using sonication in $1\times$ PBS/1% Triton X-100/protease inhibitor cocktail.

Antibodies, immunoprecipitations, and immunofluorescence
The PRMT5 and PRMT7 antibodies were obtained from Upstate Biotechnology. The SMN antibody (clone 7B10) was a gift from U. Fischer (University of Wuerzburg, Wuerzburg, Germany). The tubulin monoclonal antibody used as a loading control was obtained from Sigma-Aldrich. The methylation status of the depleted lysates was analyzed using Sym10 (Upstate Biotechnology), Sym11 (Upstate Biotechnology), and Y12 anti-sDMA antibodies (gift from J. Steitz, Yale, New Haven, CT). CFP-SmB was immunoprecipitated from cytoplasmic lysates using polyclonal GFP antibodies (Abcam). The precipitates were probed using SMN and GFP monoclonal antibodies (Roche). In the pulse-chase experiments, the newly synthesized snRNPs were precipitated using anti-TMG antibody–coated beads (Calbiochem). For the Sm core assembly assay, CFP-SmB was immunoprecipitated using the polyclonal GFP antibody. The distribution of coilin was examined using a previously generated polyclonal antibody R124 (Shpargel and Matera, 2005) and a goat anti–rabbit AlexaFluor594 secondary antibody (Jackson ImmunoResearch Laboratories). The localization of fibrillarin was determined using the 72b9 antibody (gift from E. Chan, University of Florida, Gainesville, FL), and the distribution of SMN was verified using the 7B10 monoclonal antibody. A goat anti–mouse FITC antibody (Jackson ImmunoResearch Laboratories) was used to detect fibrillarin and SMN.

Western blots and autoradiography film were quantified using the Quantity One program (Bio-Rad Laboratories).

Imaging
Immunofluorescence images were captured using a microscope (DM6000; Leica) interfaced with Volocity software (Improvision). Images were captured at room temperature using a $63\times$ oil immersion objective (Leica). The image stacks were deconvolved using Volocity software. Images were cropped using Photoshop 7.0 (Adobe).

Pulse-chase assays
The [^{35}S]methionine and [^{35}S]cysteine pulse-chase assay was performed as described previously (Winkler et al., 2005) with a few modifications. The chase time was reduced from 1 h to 30 min. The newly synthesized snRNPs were purified using anti-TMG antibody–coated beads. Cells were treated with 750 µM MTA for 24 h. An equivalent volume of DMSO was added to the control cells. In the snRNP import experiment, nuclear and cytoplasmic fractions were prepared subsequent to the pulse chase using the N-PER kit (Pierce Chemical Co.). Subsequently, each fraction was incubated with anti-TMG–coated beads. The ^{32}P-labeling experiment was performed by first depleting cells of intracellular phosphate by growing in 3 ml phosphate-free DME media for 2 h. Subsequently, 100 µCi [^{32}P]orthophosphate (PerkinElmer) was added to the cells. The cells were labeled for 2 h and harvested. For the Sm core assembly reaction, HeLa cells stably expressing CFP-SmB were used. The tagged Sm protein was immunoprecipitated using polyclonal GFP antibodies. The associated snRNAs were then examined using autoradiography. The ^{32}P-labeled cells were also used in a separate TMG-capping assay. The TMG-capped snRNPs were purified using the anti-TMG antibody–coated beads. The RNA was extracted and examined using autoradiography.

Online supplemental material
Fig. S1 shows that PRMT5 and PRMT7 associate with different proteins in vivo. Fig. S2 shows that residual snRNPs assembled under conditions of MTA treatment contain symmetrically dimethylated Sm proteins. Fig. S3 shows that SMN, PRMT5, and PRMT7 are required for TMG capping. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200702147/DC1.

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